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Original Article

Association of two polymorphisms within and near *SOCS3* gene with obesity in three nationalities in Xinjiang province of China

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Aim: *SOCS3* gene plays an important role in the pathogenesis of obesity in animal models, but the data from human studies are relatively limited. To address this issue, a genetic association analysis on nationalities with different genetic background living in the similar environmental conditions was performed.

Methods: Two thousand seven hundred eleven subjects were randomly recruited from the Kazakh, Uygur and Han nationalities in Xinjiang of China. SNP polymorphisms rs4969168 and rs9892622 within or near the *SOCS3* gene were genotyped using TaqMan-MGB™ assay. Association study between the two polymorphisms and obesity-related traits (body mass index [BMI]; waist-to-hip ratio [WHR]; weight; height, WAIST, and HIP measurements) was conducted.

Results: Significant association was found between rs4969168 and the obesity-related traits, including BMI (25.32±3.49 kg/m^2 for AA, 24.60±3.70 kg/m^2 for AG, 24.39±3.42 kg/m^2 for GG, P=0.042), weight (65.58±11.42 kg for AA, 63.50±11.30 kg for AG, 62.00±10.78 kg for GG, P=0.011) in the Han nationality, but not in the Kazakh or Uygur nationalities. Rs9892622 was significantly associated with BMI, WHR, and WAIST in the Uygur males. Rs9892622 was also associated with BMI in Kazakh males. Linear regression analysis verified the above findings. However, neither of the two polymorphisms was associated with obesity-related traits in the total population.

Conclusion: The polymorphism rs4969168 within or near the *SOCS3* gene has a significant effect in the Han nationality, while rs9892622 was associated with obesity in Uygur and Kazakh nationalities in Xinjiang of China.

Keywords: obesity; *SOCS3*; polymorphisms; rs4969168; rs9892622; China nationality

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Introduction

Obesity is a major public health problem worldwide and affects individuals across all age groups and ethnicities[1]. According to the World Health Organization (WHO), there are up to one billion overweight individuals worldwide, of which 300 million would be classified as clinically obese (www.who.int). Moreover, the situation in most developed countries is more severe. Obesity is a leading risk factor for many health problems such as heart disease, type 2 diabetes and most cancers[2]; furthermore, it accounts for up to 7.8% of the total health care expense in developed countries[3]. Numerous studies have attempted to illustrate the pathogenesis of obesity.

A multitude of genes associated with obesity have been discovered, with one of the most important being *SOCS3* (suppressor of cytokine signaling 3), which is located in the chromosome region 17q24-17q25. *SOCS3* works as a feedback inhibitor for a range of cytokine signals and inhibits the function of leptin and downstream steps in the insulin signaling pathway to regulate energy balance[4–7]. Although this function has been confirmed in animal models, the association data from human studies are relatively limited. Talbert *et al* have shown that the *SOCS3* gene is related to body mass index (BMI), visceral adipose (VAT), and waist circumference (WAIST) in Hispanic families[8]. However, in the study performed by Jamshidi *et al*[9] on the association analyses of common polymorphisms (rs4969169, rs12953258, and rs8064821) in *SOCS3* between two normal female twins that examined body weight, insulin sensitivity or lipid profile, none of the three polymorphisms were found to be associated with obesity. In addition, the study on the association analysis of two polymorphisms in the coding sequence and promoter region of the

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SOCS3 gene (including -920 C>A (rs12953258) and -1044 C>A) in German children and adolescents with extreme obesity suggested that no association was observed[10].

This report focuses on the association between the *SOCS3* gene and obesity-related traits in the Kazak, Uygur and Han nationalities from the Xinjiang province of China. Two SNP polymorphisms were selected according to the data from tag-polymorphisms of the HapMap project and previous studies[11, 12]. One polymorphism is the A+930→G (rs4969168) polymorphism that is located in the 3′UTR of the *SOCS3* gene, which is a haplotype tagging SNP (htSNP) that sufficiently covers the genetic variation of the entire gene. The other SNP selected for this study is rs9892622 A→G, a high-frequency variant near the gene, which is located in the upstream promoter region of the *SOCS3* gene and appears to be a reasonable functional candidate for the *SOCS3* locus and obesity.

Materials and methods

Subjects

Subjects (n=2711) were recruited from three races of Xinjiang in China. All subjects passed the normal medical examination and were selected randomly, including 1045 Kazakh, 804 Uygur and 862 Han individuals within the age range of 19–87 years old. The local ethics committee approved the study, and all participants provided informed consent. Standing height (height) was measured (to the nearest 0.01 m) with a stadiometer. Body weight (weight) was measured (to the nearest 0.01 kg) with a weighing machine. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Lastly, waist circumference (WAIST), hip circumference (HIP), and waist to hip ratio (WHR) were determined as previously described[13].

DNA preparation

Genomic DNA was extracted from peripheral blood leukocytes of all samples by the phenol-chloroform nucleic acid extraction method. The genomic DNA concentrations of samples were measured by using a NanoDrop Spectrophotometer (Thermo Fisher, Boston, MA, USA) and were qualified for analysis when the ratio of wavelengths 260/280 ranged between 1.7–2.0. Next, genomic DNA samples were diluted to 10 ng/μL as the working concentration.

Polymorphisms selection and genotyping

Two SNP variants rs4969168 and rs9892622 were selected. Rs4969198 was selected as a tagSNP to cover all of the related polymorphisms on *SOCS3* that are based on the recently released HapMap data. Because common variants are susceptible to common diseases, we chose another potential functional locus with a relatively high frequency, rs9892622, in the promoter region of *SOCS3*. Genotyping was performed using the 5′-nuclease TaqMan-MGB™ assay in 384-well plates and the ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA, USA). The raw data were read at the end-point and were clustered into three groups that represented the three genotypes.

Statistical analysis

The data were analyzed with SPSS (version 13.0). Before any other analysis, the maximum likelihood estimate of allele frequencies was tested for departures from Hardy-Weinberg Equilibrium proportions (HWE) using a chi-squared goodness of fit test to make sure that there were no typing errors or bias from population structure. The distribution of obesity-related quantitative traits (BMI, height, weight, WHR, WAIST and HIP), characteristic variants (age, gender, *etc*) and genotypes among the three nationalities were analyzed by one-way ANOVA with SNK test. A *t*-test (independent samples) was used to investigate the distribution of variants in different genders. Stratified analysis by nationality and gender was performed to explore the interaction between environmental and genetic factors. Additionally, linear regression analysis was used to determine the importance of obesity-related traits. To avoid over-correction induced by measuring too many obesity-related traits, only the variants of age, nationality, gender and genotypes were involved in the regression analysis. The *P*-value confidence level was set at 0.05 for the above tests.

Results

Sample characteristics

After removing samples with missing data, a total population with n=2667 samples was left, which comprised the three races, Kazakh, Uygur and Han; the number of samples from each race and their percentage of the total sample population were 1017 (38.14%), 798 (29.92%), and 852 (31.94%), respectively. The distribution of sample characteristics (mean±SD of obesity-related traits and distribution of genotypes of SNP polymorphisms) in each nationality are displayed in Table 1. The statistical differences of these variants among nationalities were determined by the Fisher LSD test. The data showed that all of the variants (age, BMI, weight, height, WHR, WAIST, HIP, rs4969168, and rs9892622) were statistically different among the three nationalities and indicated that analysis of the interaction of gene polymorphisms and environmental factors in a total population would be inappropriate; rather, a separate analysis of the effects of interaction within individual populations of each nationality would be more appropriate.

The distribution of variants, except polymorphisms, in different genders was also statistically analyzed by independent samples in a *t*-test (Table 2). It was found that the variants, except WHR, were significantly different between males and females. The distributions of genotypic polymorphisms in different genders were analyzed by a χ^2 square test; no difference was found in the genotypic frequencies between genders, which indicated that no typing bias or error existed.

Genotyping and allele frequencies

The genotyping success rates of both rs4969168 and rs9892622 were 97.4%. The allele frequencies of *SOCS3* rs4969168 and rs9892622 for the total population and each subpopulation (classified by race) were collected (Table 1). In the Kazakh, Uygur and Han subpopulations, the allele frequencies of rs4969168 were 0.582, 0.625, and 0.572, respectively, and the

Table 1. Sample characteristics.

	Total (2658)	Kazakh (1016)	Uygur (798)	Han (844)	*P*
Gender (M/F)	1062/1596	434/582	294/504	334/510	0.039
Age	50.86±11.52	47.9±10.5	52.7±10.3	52.6±12.9	<0.001
BMI (kg/m^2)	25.68±4.19	25.64±4.18	26.77±4.54	24.67±3.56	<0.001
Weight (kg)	1.61±0.09	68.64±13.07	67.90±12.76	63.48±11.16	<0.001
Height (m)	66.76±12.59	1.63±0.09	1.59±0.08	1.60±0.08	<0.001
WHR (cm/cm)	0.880±0.073	0.90±0.73	0.88±0.08	0.87±0.07	<0.001
WAIST (cm)	86.57±11.98	91.95±12.59	88.15±11.69	82.64±10.59	<0.001
HIP (cm)	98.26±9.51	102.21±10.09	100.50±9.43	94.37±7.75	<0.001
rs4969168 (*n*/%)	2597	996 (100)	787 (100)	815 (100)	0.001
AA	415 (16.0)	159 (16.0)	115 (14.6)	142 (17.4)	
AG	1301 (50.1)	514 (51.6)	360 (45.7)	427 (52.4)	
GG	881 (33.9)	323 (32.4)	312 (39.6)	246 (30.2)	
rs9892622 (*n*/%)	2598 (100)	990 (100)	778 (100)	830 (100)	0.009
AA	555 (21.4)	294 (29.7)	198 (25.4)	238 (28.7)	
AG	1313 (50.5)	495 (50.0)	384 (49.4)	434 (52.3)	
GG	730 (28.1)	201 (20.3)	196 (25.2)	158 (19.0)	

Different population sizes between some variants and total were due to different samples of missing data, which was the incomplete data for rs4969168 or rs9892622. The missing data was excluded with default settings during the analyses.

Table 2. Distribution of variants in different genders.

	Male	Female	*P*
Age	52.70±11.7	49.63±11.21	0.004
BMI	25.39±3.83	25.86±4.40	<0.001
WHR	0.89±0.07	0.87±0.07	<0.001
Weight	71.40±12.22	63.71±11.89	<0.001
WAIST	87.60±11.92	85.95±11.98	0.003
Height	1.68±0.07	1.57±0.06	<0.001
HIP	97.75±9.39	98.56±9.58	0.064
rs4949168	1062 (100%)	1596 (100%)	0.639
AA	158 (14.9%)	256 (16.0%)	
AG	529 (49.8%)	769 (48.2%)	
GG	350 (33.0%)	528 (33.1%)	
Missing[a]	25 (2.3%)	43 (2.7%)	
rs9892622	1062 (100%)	1596 (100%)	0.405
AA	304 (28.6%)	424 (26.6%)	
AG	515 (48.5%)	793 (49.7%)	
GG	212 (20.0%)	342 (21.4%)	
Missing[a]	31 (2.9%)	37 (2.3%)	

[a] Missing value was the number of population with incomplete data for rs4969168 or rs9892622, and it was excluded with default settings during the analyses.

allele frequencies of rs9892622 were 0.453, 0.499, and 0.452, respectively. There was no departure from the Hardy-Weinberg equilibrium in any of the groups (P>0.05). One-way ANOVA was used to determine that the distribution of polymorphisms was statistically different among the three nationalities.

Association of the *SOCS3* gene with obesity-related quantitative traits

Overall analysis

The association of the two polymorphisms (rs4969168 and rs9892622) with BMI, WHR, height, weight, WAIST, and HIP was determined by one-way ANOVA with SNK test, but no evidence of association was found between each of the two polymorphisms and the above quantitative traits in the total population.

Stratified analysis of nationality

Because obesity is due to the interaction of environmental and genetic factors and the above data showed differences between nationalities or gender regarding obesity-related traits, it was supposed that environmental background, race, gender or age might have a significant interaction with the polymorphisms, which may lead to obesity or related traits. Therefore, stratified analysis was performed based on the interaction between nationalities and polymorphisms. Although no significant difference was observed among the three genotypes of rs4969168 in the total population, the data indicated that a remarkable difference existed within the subpopulations (Table 3 and 4). Significant differences were found in the mean of BMI, weight, and WAIST among the pair-wise comparison of the genotypes of rs4969168. Although most compared pairs were not found to be statistically significant, there were noticeable trends (Table 3). Based on the data from the Han group, as the frequency of the G allele increased, the BMI, weight and WAIST measurements decreased, which suggests that the G allele may play a role in maintaining a reduced body weight in the Han group. However, the opposite trend was seen in the Kazakh

Table 3. Mean of BMI, Weight, WAIST and WHR of each genotype of rs4969168 in each nationality.

rs4969168	Genotype (%)	BMI	Weight	WAIST	WHR
Kazakh	GG (32.4)	25.70±4.21	68.75±12.83	93.30±11.77	0.91±0.08
	AG (51.6)	25.62±4.25	68.88±13.38	91.74±12.58	0.90±0.07
	AA (16.0)	25.51±3.91	67.73±12.51	89.22±14.01	0.88±0.08
Uygur	GG (39.6)	26.75±4.55	68.04±12.72	88.40±11.49	0.88±0.08
	AG (45.7)	26.77±4.63	67.76±13.02	87.84±12.06	0.88±0.07
	AA (14.6)	26.95±4.31	68.25±12.45	88.71±11.01	0.87±0.07
Han	GG (30.2)	24.39±3.42	62.00±10.78	82.11±9.90	0.87±0.07
	AG (52.5)	24.60±3.70	63.50±11.30	82.46±11.03	0.87±0.07
	AA (17.3)	25.32±3.49	65.58±11.42	84.18±10.32	0.88±0.06

Table 4. *P*-value of pairwised comparison among genotypes of rs4969168.

rs4969168		BMI	Weight	WAIST	WHR
Kazakh	Total	0.900	0.622	0.078	0.115
	Male	0.486	0.362	0.363	0.277
	Female	0.617	0.630	0.094	0.242
Uygur	Total	0.925	0.924	0.763	0.732
	Male	0.907	0.695	0.663	0.965
	Female	0.996	0.946	0.917	0.503
Han	Tatal	**0.042**	**0.011**	0.361	0.157
	Male	0.044	0.054	0.260	0.752
	Female	0.361	0.352	0.957	0.712

Table 6. *P*-value of pairwised comparison among genotypes of rs9892622.

rs9892622		BMI	WEIGHT	WAIST	WHR
Kazakh	Total	0.058	0.169	0.072	0.137
	Male	**0.025**	0.075	0.143	0.151
	Female	0.535	0.454	0.338	0.471
Uygur	Total	0.852	0.842	0.336	0.594
	Male	**0.049**	**0.040**	0.103	**0.045**
	Female	0.072	0.137	0.310	0.774
Han	Tatal	0.249	0.364	0.919	0.672
	Male	0.549	0.853	0.775	0.905
	Female	0.285	0.379	0.939	0.585

group but not in the Uygur group. The potential cause of this trend will be further covered in the latter part of the discussion.

For rs9892622, a significant difference for obesity-related traits was not observed in the total population. Statistically meaningful results only appeared in the male subpopulation of the Uygur group, but no obvious trend could be summarized from the datasets (Table 5 and 6).

Linear regression analysis

Linear regression analysis confirmed the previous results in which nationality and gender were included, but age and the two polymorphisms excluded, in the model with $P<0.05$. In the following stratified analysis by nationality or gender, the two polymorphisms were also included with $P<0.05$, but age was excluded from the model. Because nationality seemed to be the most effective factor in our data, we performed a linear regression analysis for each nationality. In the Han population, gender was the first factor involved in the regression ($P=0.004$), followed by rs4969168 ($P=0.015$).

Discussion

With the release of data from the HapMap phase III project and completion of several whole genome association studies

Table 5. Mean of BMI, Weight, WAIST, and WHR of each genotype of rs9892622 in each nationality.

rs9892622	Genotype (%)	BMI	Weight	WHR	WAIST
Kazakhi	GG (20.3)	25.20±3.94	67.23±12.11	0.88±0.06	89.49±12.13
	AG (50.0)	25.96±4.18	69.31±13.09	0.91±0.08	92.82±12.80
	AA (29.7)	25.41±4.35	68.45±13.61	0.90±0.07	92.56±12.65
Uygur	GG (25.2)	26.91±5.15	67.75±13.58	0.87±0.08	87.98±12.42
	AG (49.4)	26.70±4.16	68.10±12.06	0.88±0.08	87.80±11.47
	AA (25.4)	26.69±4.63	67.45±13.39	0.88±0.07	88.85±11.56
Han	GG (19.0)	24.24±3.49	62.30±11.10	0.87±0.07	81.96±10.80
	AG (52.3)	24.78±3.60	63.70±11.39	0.87±0.07	82.83±11.01
	AA (28.7)	24.75±3.56	63.72±10.70	0.87±0.07	82.46±9.70

on several common diseases, scientists realized that tagSNPs in target genes could be selected as candidate loci to cover all of the polymorphisms involved in the haploblocks of the target gene.

SOCS3 is one of several genes involved in cytokine signaling and regulates the function of proteins downstream, inhibits the insulin signaling pathway and stimulates the upregulation of TNFα in adipose tissue[14]. The protein encoded by *SOCS3* is also important in energy balance and regulation[4–7]. This result has been confirmed in animal models, but the association data in humans have been relatively limited. Research by Talbert *et al* has shown that the *SOCS3* gene is related to body mass index (BMI), visceral adipose (VAT), and waist circumference (WAIST) in Hispanic families[8]. However, in a study reported by Jamshidi[9] on the association analyses of common polymorphisms (rs4969169, rs12953258 and rs8064821) in *SOCS3* with body weight, insulin sensitivity or lipid profile in normal female twins, none of these three polymorphisms was found to be associated with obesity. In a previous study, an association analysis of two polymorphisms (-920 C>A (rs12953258) and -1044 C>A) was performed, one in the coding sequence and another in the promoter region, in the *SOCS3* gene in German children and adolescents with extreme obesity[10]. As a result, there was no association observed between the two polymorphisms in *SOCS3* and extreme obesity. Weight is a complex phenotype that is affected by several biological pathways; the most important two pathways are the neural control system of food intake and energy balance regulation. Thus, one explanation to these ambivalent results is that the pathogeny of obesity differs among populations.

Another potential explanation is that different haploblock distributions appear in different ethnic groups or in different nationalities. Consequently, pathogenic loci linked to tagSNPs can be partially or totally different, and in some cases, special ethnic tagSNPs would lose their ability of catching pathogenic loci in other ethnic studies, which seems to have happened in our study. Our data suggest that there is no association between rs4969168 and obesity-related traits in the total population (P>0.05). However, a significant association was observed for BMI and weight in the Han population and male subgroup, which indicates that the polymorphism rs4969168 seems to have a different association effect on different nationalities and different genders. In addition, the data showed that the allele A of rs4969168 was associated with increased BMI and weight in the Han group. Moreover, the population with the AA genotype of rs4969168 is more susceptible to weight-gain in the Han population, especially in males. However, a totally opposite result was discovered in the Kazakh population, which indicates that the mechanisms may likely be distinct between these two nationalities. Our results are reasonable given that *SOCS3* serves as both a pivotal and hub function in a complex regulatory system, which further suggests that *SOCS3* may also play a dual role in weight control.

Similar to rs4969168, there was no evidence for an association between rs9892622 and obesity-related traits in the total population, but a significant association appeared in the stratified analysis. Interestingly, distinct from rs4969168, rs9892622 was associated with BMI, weight and WAIST in the Kazakh and Uygur populations rather than the Han population.

In conclusion, whether weight control is attributed to different pathogenic factors or the distribution of ethnic haploblocks, the genetic background seems to certainly play a basic role. According to our current data, both rs4969168 and rs9892622 are associated with obesity, and the association effect is different according to nationality and gender. Further study is required to fine-map the pathogenic loci and discover the functional impact of *SOCS3* in obesity.

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Author contribution

Wei TANG designed the research, executed the experiments and wrote the paper; Yong-quan SHI and Zhi-min LIU designed the research and wrote the paper; Jun-jie ZOU and Jiao-yang ZHENG contributed analytic tools; and Xiang-fang CHEN and Hua-zong ZENG analyzed the data.

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